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The influence of certain environmental factors on the respiration of some plant-parasitic nematodes.

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THE INFLUENCE OF CERTAIN ENVIRONMENTAL FACTORS ON THE RESPIRATION OF SOME PLANT-PARASITIC NEMATODES

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CHAPTER I

INTRODUCTION

The study of plant-parasitic nematodes began in 1743 when Needham discovered the cryptobiotic nature of Anguina tritici larvae inside wheat galls. Since that time, a small but active group of workers, primarily taxonomists and plant pathologists, have studied nematodes parasitic on plants. By the late 1940's enough was known about the extent of damage done by nematodes for plant nematology to emerge as a distinct discipline. Most of what is known about plant-parasitic nematodes, however, has been discovered in the last twenty years. The goal before the plant pathologist has been to control the nematodes causing disease in plants, and, as a result, a great deal of information has accumulated regarding their pathogenic behavior. Very little is known at present about the physiology of plant-parasitic nematodes, especially when compared to animal parasitic nematodes.

Respiratory physiology of nematodes parasitic on animals has been extensively investigated (von Brand, 1942, 1943, 1952; Rogers, 1948, 1949a, 1949b, 1961; Schwabe, 1957; Fairbairn, 1954, 1957; Roberts and Fairbairn, 1965), whereas only a few studies have been made on the respiration of free living nematodes. Nielsen (1949) measured the rate of respiration of a number of soil nematodes for the first time using the Cartesian diver technique. He published more extensive studies on the ecology of soil-inhabiting nematodes

in 1961. He has calculated that the oxygen consumption of soil inhabiting nematodes is 600 m^3/hectare/hour, out of which 56% was due to bacterial feeders and approximately 20% by Tylenchida feeding largely on plant roots.

Santmeyer (1956) carried out oxygen consumption studies on the free-living nematode Panagrellus redivivus studying the effect of temperature and starvation on rate of respiration. He suggested that screening of candidate nematocides can be done in the laboratory by studying the effect of these compounds on the rate of respiration of P. redivivus.

The main reason for the small number of studies on the physiology of plant-parasitic nematodes has been the non-availability of these relatively small nematodes in large numbers and in a germ-free state. Methods devised by Mountain (1955), Tiner (1960) and Krusberg (1961) have made it possible to culture several species of plant-parasitic nematodes in quantity on plant tissue grown under aseptic conditions. The majority of plant-parasitic nematodes, however, have defied all attempts at culture in the laboratory.

There are inconclusive indications that plant-parasitic nematodes are obligately aerobic (Steiner, 1952). Nielsen (1949, 1961) concluded from Cartesian diver measurements that a 10% decrease in oxygen tension did not affect the rate of respiration of soil nematodes. Feldmesser and Feder (1954) and Feder and Feldmesser (1955) carried out qualitative studies on the effect of lowered oxygen

tension on some free-living and plant-parasitic nematodes. They reported that motility of these nematodes was reduced at low oxygen tensions and that different species had different tolerance levels to lowered oxygen tensions. Investigations of Stolzy, et al. (1960), van Gundy and Stolzy (1961, 1963), and van Gundy et al. (1962), revealed that activity and survival of those plant parasitic nematodes studied was related to oxygen diffusion rates in the soil pore spaces and to oxygen concentrations applied over the soil surface.

Rohde (1960) measured oxygen consumption of a number of plant-parasitic nematodes for the first time. He investigated the influence of starvation and different concentrations of carbon dioxide on the respiratory rates of Pratylenchus penetrans and Hoplolaimus tylenchiformis. Wallace and Greet (1964) reported the effect of increasing osmotic pressure on respiration and motility of the drought resistant nematode Tylenchorhynchus icarus.

Effect of moisture on the metabolic rate of drought-resistant nematodes. Although a certain amount of moisture is essential for the survival of most plant-parasitic nematodes, some species can tolerate almost complete desiccation. Peacock (1957) reported that there is an optimum soil moisture level for the survival of root knot nematodes, below which populations die out. Tolerance to desiccation varies greatly, however, in different plant-parasitic nematode species (Wallace, 1963; van Gundy, 1965).

Both larvae and adults of two species of fresh-water nematodes, Actinolaimus hintoni and Dorylaimus keilini, are able to resist desiccation (Lee, 1961). Among the plant-parasitic nematodes, Anguina tritici (second stage larvae), Anguina agrostis (second stage larvae), Ditylenchus dipsaci (fourth stage larvae) and Aphelenchoides ritzemabosi (adults) are known to survive desiccation for many years. A. tritici second stage larvae have been revived from wheat galls which had been stored for 28 years (Fielding, 1951). Similarly, second stage larvae of A. agrostis have been recovered from bentgrass seed galls after ten years, and fourth stage larvae of D. dipsaci after 23 years of storage in dry lily bulbs (Fielding, 1951). While desiccated, these drought-resistant species are inactive and their metabolism is considered to be at a very low level, although the exact level of their metabolic activities is unknown and little is known regarding the degree of desiccation of their bodies (van Gundy, 1965).

Various names have been applied to the inactive, desiccated state --dormancy, anabiosis, abiosis. Keilin (1959) devised new terms to indicate different states of lowered metabolism, which he called hypobiosis. The inactive state may be further classified, according to Keilin, as cryptobiosis, which means a complete suspension of all reversible life processes for considerable periods of time. When caused by desiccation, the inactive state is further described as anhydrobiosis. The term anhydrobiosis best describes the inactive state of the

plant-parasitic nematodes.

The mechanism of survival of nematodes during dehydration is unknown (van Gunday, 1965), and no special organs in the nematode body are known to be involved in survival during this period.

The effect of osmotic pressure and composition of the medium on the rate of respiration. Very little work has been done on the osmotic relationships of nematodes as compared to other invertebrates. Animal-parasitic nematodes can withstand only osmotic pressure near that of the host tissue, which is generally equivalent to 0.87% sodium chloride solution and relatively constant (von Brand, 1952). The respiratory rate of larval Eustrongyloides ignotus remained unaltered in solutions varying from distilled water to 1.5% sodium chloride, while 2% and 4% sodium chloride solutions led to a progressive decline in oxygen consumption (von Brand, 1942).

As von Brand (1960) has stated, "The experimental analysis of the osmotic relationships of plant-parasitic nematodes has not progressed very far." Plant-parasitic nematodes must tolerate the osmotic range of the host tissue, which itself is very wide in a single plant. Nematodes living in soil have to face variations of osmotic stress resulting from rain and drying of soil (Lee, 1965). Wallace (1963) has reported that most plant-parasitic nematodes can tolerate osmotic pressures up to ten atmospheres, whereas in most agricultural soils osmotic pressure rarely exceeds two atmospheres (Russell,

1950). Van Gundy (1965) has reported that osmotic tolerance of some species of nematodes, as exhibited by ability to move, varies from 4.48 atmospheres to 22.5 atmospheres.

Osmotic pressures of 22.4 atmospheres and higher have been reported to be harmful to plant-parasitic nematodes. Blake (1961) reported that fourth stage larvae of D. dipsaci were killed in 1 M (22.4 atmospheres osmotic pressure) and 2 M (44.8 atmospheres osmotic pressure) urea solutions. Feder (1960) found that 1% to 5% sucrose or dextrose by weight of soil was nematicidal and proposed this as a method for controlling nematodes in the field. Such a method has great practical disadvantages.

Machmer (1958) reported that elevated osmotic pressure had a stimulatory effect on Tylenchulus semipenetrans and Meloidogyne incognita acrita. He found that populations of these nematodes increased with high soil salinities.

In very dry soils and desiccated plant tissue such as dry bulbs, leaves and seeds, exosmosis of fluids from the nematode body may occur. This desiccated state may help them to tolerate extremes of temperature. Bosher and Mckeen (1954) found that D. dipsaci in the dried quiescent state survived -80°C for 20 minutes, but the nematodes were killed if they were subjected to this temperature when wet. Second stage larvae of Anguina tritici inside wheat galls withstood dry heat (55°C) for two hours, whereas larvae in galls died in 25

minutes when heated to this temperature in water (Bloom, 1963).

Wallace and Greet (1964) used urea to study the effect of osmotic pressure on Tylenchorhynchus icarus. They have not mentioned the toxicity of urea to this nematode. The maximum motility of this nematode was in 10^{-3} M urea solution, but in 1 M urea nematodes were not motile. They found that its oxygen consumption was high between 10^{-3} M and 1 M urea solutions, but was low in 2 M urea solution. They concluded that the capacity of T. icarus to resist desiccation is reflected in the stimulation of respiration by high osmotic pressure, which in nature accompanies drought.

Potassium and sodium ions have been reported to have different effects on rate of oxygen consumption of nematodes (von Brand, 1952). In larval Eustrongyloides ignotus, the rate of oxygen consumption was increased in potassium chloride solution; the rate being about twice that found in an isotonic sodium chloride solution (von Brand, 1943). Bueding (1949), on the other hand, found in Litomosoides carinii that exclusion of sodium chloride produced a marked decrease in the filarial metabolism and motility, even when the ionic strength of the medium without sodium chloride was the same as with it. Moreover, when the medium contained high concentrations of potassium instead of sodium, metabolic activity and motility of the worm decreased markedly. Roberts and Fairbairn (1965) measured the rate of respiration of Nippostrongylus brasiliensis in a medium containing 127 mM sodium chloride and 5 mM potassium chloride. When they reversed the concentration of sodium

with potassium, the respiratory rate did not change.

Effect of concentration of carbon dioxide on rate of respiration. Loomis (1957), in the conclusion of his review on sexual differentiation in hydra wrote, "In general, it appears that the level of carbon dioxide in the environment of the living cell is one of the most labile and neglected of all biological variables, yet one that is capable of regulating both the rate of the cell division and the process of differentiation, rather than being merely a waste product of metabolism." Apart from its role in stimulating the respiratory center in the brain of mammals, very little is known regarding the role of carbon dioxide in influencing the respiratory pattern of animals.

Warren (1944) found that respiration of various mammalian tissues is of the order of 20% to 40% higher in bicarbonate Ringer's medium than in phosphate Ringer's medium. Likewise, Laser (1942) reported that carbon dioxide stimulated oxygen uptake by mouse kidney slides. Root (1930) found that in Paramecium caudatum, respiration was slightly increased at lower tensions of carbon dioxide, but decreased at carbon dioxide tensions above 5%. He attributed this stimulation of respiration by carbon dioxide to increased motility. He further found that oxygen uptake in fertilized eggs of Arbacia sp. was depressed by carbon dioxide at all tensions studied.

Schwabe (1957) has pointed out that the presence or absence of carbon dioxide has not been adequately considered as a factor in helminth metabolism. He found that in free-living filariform larvae of

Nippostrongylus muris, endogenous Q_{O_2} is the same in the absence or presence of 5% carbon dioxide, whereas the Q_{O_2} of parasitic filariform larvae from lungs is 18% to 65.3% lower in the absence of carbon dioxide than in its presence. The activity of exsheathment stimulus in Trichostrongylus axei and Haemonchus contortus increases with increasing concentration of undissociated carbon dioxide plus dissociated gaseous carbon dioxide (Rogers, 1960).

Carbon dioxide has been found to be fixed by some animal-parasitic nematodes. Eggs of Ascaris lumbricoides during development use carbon dioxide, which is incorporated into glycogen and trehalose (Passey and Fairbairn, 1957). $C^{14}O_2$ fixed by Heterakis gallinae, first appears in oxaloacetic acid (Fairbairn, 1954). Saz and Vidrine (1959), Saz and Hubbard (1957) and Bueding (1962) have further elaborated the mechanism of carbon dioxide fixation by nematodes.

Carbon dioxide has been proposed as one of the factors which stimulates plant-parasitic nematodes to be attracted to roots in the soil (Kingler, 1965). Kampfe (1959) found that mobility of Heterodera rostochiensis and H. schachtii was increased by previous exposure to carbon dioxide and that six days' exposure to pure carbon dioxide caused no damage to larvae, which quickly became active when oxygen is reintroduced. Wallace (1959) suggested that accumulation of carbon dioxide in cysts of H. schachtii might be the cause of incomplete hatch. Rohde (1960) proposed a hypothesis that carbon dioxide released by respiring roots acts as an orthokinetical stimulus to decrease activity and prevent nematodes from leaving the root area.

The methods generally used to measure oxygen uptake (e.g. Warburg respirometer) involve the use of alkali to remove all carbon dioxide from the environment of the organism, a situation not comparable to that which occurs in nature. Very few studies have been made in the presence of carbon dioxide and in most of these studies carbonate-bicarbonate buffer was used to maintain a definite concentration of carbon dioxide in the medium (Warren, 1944; Laser, 1942; Root, 1930; Schwabe, 1957). Such buffer systems have been criticized by Pardee (1949) and Krebs (1951) on the grounds that these buffers provide only approximate carbon dioxide concentrations and can be used only when carbon dioxide pressures are of the order of a few mm mercury. Di-ethanolamine buffer systems recommended by Krebs (1951) can be used to maintain concentrations up to 2% carbon dioxide precisely and up to 6% with some degree of reliability. Rohde (1960) made use of these buffers with the Cartesian diver technique and found that the rate of respiration of Pratylenchus penetrans and Hoplolaimus tylenchiformis is inhibited by carbon dioxide concentrations higher than that of air, but a carbon dioxide concentration equal to that in air appears to have a stimulatory effect on respiration.

Effect of temperature on rate of respiration. The optimum temperature for most functions of plant-parasitic nematodes lies in the range of 15° - 30°C (Wallace, 1963). Optimum temperature ranges for different activities such as motility, reproduction and larval emergence may be different even for a single species. Moreover, the optimum

temperature for a species may be different in different plant hosts, as was shown by Krusberg (1959) for Tylenchorhynchus claytoni. This species has an optimum temperature range for reproduction of 21° - 27°C on wheat and 29° - 35°C on tobacco. Such differences may be due to changes in the host plant itself as affected by temperature.

Krogh (1916) established a 'Normal Curve', applicable to a wide variety of animals, obtained by plotting respiratory rates against temperature. Respiratory rates of larval Eustrongyloides ignotus (von Brand, 1943) and Mononchus papillatus (Nielsen, 1949) fall along the normal curve of Krogh. The respiratory rates of Panagrellus redivivus obtained by Santmeyer (1956), however, do not group along this normal curve.

In addition to the normal curve of Krogh, the temperature-respiratory rate relationship has also been expressed in terms of Q_{10} and the Arrhenius' equation. Q_{10} is the ratio of the velocity constant of a process or a reaction at a given temperature to the velocity constant at a temperature 10°C lower.

The application of the Arrhenius' equation to biological processes was popularized by Crozier (1926a, 1926b). This equation may be expressed as:

$$\mu = \frac{R\,(\log_e K_2 - \log_e K_1)}{\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$$

where:

μ is the temperature characteristic or thermal increment

R is gas constant

K_1 and K_2 are the rates of biological process at absolute temperatures T_1 and T_2 respectively.

The increase in rate of respiration with increase in temperature is in accordance with the Arrhenius equation if the plot of the log of respiration rate against the reciprocal of absolute temperature is a straight line. Sometimes the points fall along two or, rarely, more intersecting lines, each straight line representing a different temperature characteristic. The point of intersection of these lines represents a shift in the master, or "bottle-neck" reaction which limits the overall process of biological phenomenon, in this case respiration (Crozier, 1926a).

OBJECTIVES.

The purpose of the present investigation was to study the effect of different environmental factors, -- moisture, osmotic pressure, ionic composition of the medium, nutrients, carbon dioxide concentration, and temperature on the rate of respiration of some plant-parasitic nematodes and to correlate the findings with the conditions in nature under which these animals survive. Special attention has been given to the study of cryptobiosis in Anguina tritici and A. agrostis.

CHAPTER II

MATERIALS & METHODS

Materials. Second stage larvae (L_2 larvae) of Anguina tritici (Steinbuch, 1799); Chitwood, 1935) were obtained from ten-year-old wheat galls and from galls harvested in 1965. Second stage larvae of Anguina agrostis (Steinbuch, 1799; Filipjev, 1936) were obtained from bent grass seed galls harvested in 1965. Adults of Pratylenchus penetrans (Cobb, 1917; Chitwood & Oteifa, 1952), Ditylenchus dipsaci (Kuhn, 1857; Filipjev, 1936) and Aphelenchoides ritzemabosi (Schwartz, 1911; Steiner, 1932) were obtained from monoxenic cultures maintained on alfalfa callus tissue growing in nutrient agar (Krusberg, 1961).

Galls of wheat were surface sterilized with 2.5% sodium hypochlorite for five minutes and bentgrass seed galls for one to two minutes and thoroughly rinsed with sterile distilled water several times. To obtain larvae, the galls were opened in sterile distilled water. Larvae were then treated with a solution of 0.05% dihydrostreptomycin sulfate (W/V) and 0.05% neomycin sulfate (W/V) for five minutes and were finally rinsed with sterile distilled water.

To obtain nematodes from cultures, infected alfalfa callus tissue was placed on a cotton plug in the upper part of a 15 ml centrifuge tube filled with enough water to wet the plug. The tube with water and the plug had been previously capped with aluminum foil and autoclaved. The nematodes settled to the bottom of the tube within a few hours.

Nematodes were always stored at 5° - 10°C to check microbial growth

and reduce nematode motility. Unless otherwise noted, infected callus tissue was placed in the centrifuge tube for nematode extraction 20 hours before respiration measurements were made.

Respirometry. Respiration was measured by three methods: The Cartesian diver technique, the Warburg direct method and Gilson's differential method. The Gilson differential respirometer is slightly more sensitive than the Warburg manometer whose sensitivity is about 1 µl (Giese, 1963). The Cartesian diver technique is about 1000 times more sensitive than either the Warburg or Gilson method.

For most experiments, a Cartesian diver microrespirometer, similar in design to that of Linderstrøm-Lang (1943) and Holter (1943) was used (Fig. 1). Divers with a total volume of 8 - 15 µl were constructed from pyrex glass capillaries. Five to ten nematodes were placed in 0.5 - 1.00 µl of medium in the bulb of each diver and neck seals were added. (Fig. 2). Five divers with nematodes and a blank diver were run simultaneously for each treatment. Nematodes were counted and checked for motility before the divers were placed in the flotation vessels as well as after the experiment was over. The readings for a diver were discarded, if part or all the nematodes were not recovered live.

With the Warburg and Gilson respirometers, 7 ml flasks were used. Each flask received 8,000 to 180,000 nematodes and two to five replications were arranged for each determination. The shaking rate of the flasks was 95 per minute, with a stroke length of 4.5 cm.

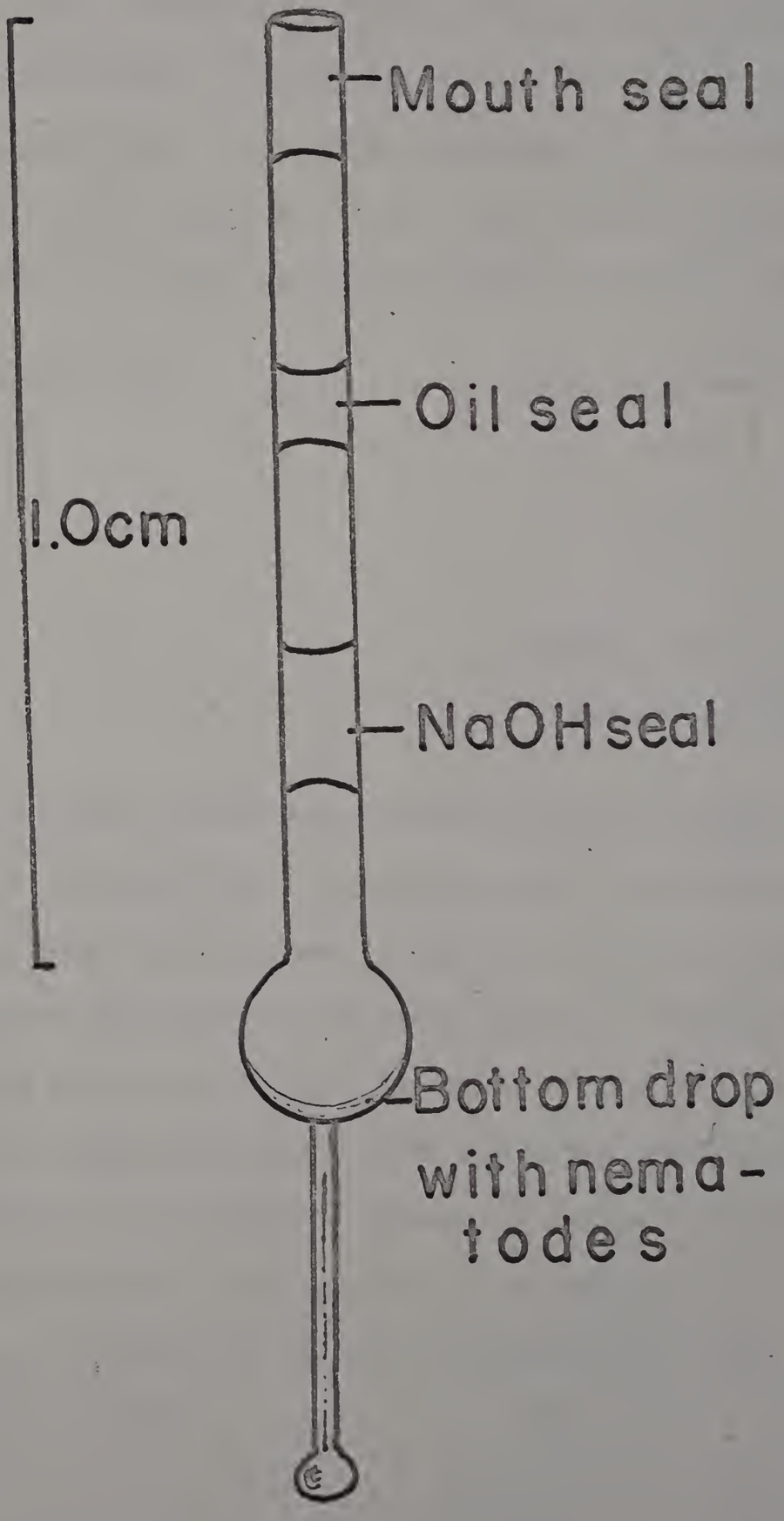
Except for studies when temperature was deliberately varied, all measurements were made at 22° ± 0.01 C.

Nematodes, glassware and media were essentially aseptic at the beginning of each experiment. In addition, a blank was run with each experiment containing the same medium from which nematodes had been removed. The blank showed gas consumption very rarely; if it did, the experiment was discarded. Contamination was also detected by plating the nematode medium. For this, one ml of the medium (usually water), which might include some nematodes also, was mixed with 10 ml of potato dextrose agar at 35°C, and then poured in a dish. The dish was stored at 37°C. If more than 50 colonies were counted after 48 hour incubation, the experiment in which these nematodes were used was discarded. Due to limitations in working with large numbers of divers in the Cartesian diver respirometer and non-availability of nematodes in large numbers for other methods, control vessels were not included; however, emphasis was given to replications and determinations on more than one day.

Rate of respiration (Q_{O_2}) has been expressed as μl oxygen/μg dry weight/hour (=μl oxygen/mg dry weight/hour = ml oxygen/gram/hour). Each Q_{O_2} value has been calculated from one to three determinations of at least three replications each. Dry weight was determined for each species both as suggested by Roberts (1961) and by Myers and Krusberg (1965). For each dry weight determination, 1,000 to 15,000 nematodes were used. Each dry weight determination was repeated five times.

Fig. 1. Cartesian diver Microrespirometer.

Fig. 2. A fully loaded Cartesian diver.


Mouth seal
Oil seal
1.0cm
NaOH seal
Bottom drop with nema-todes

Effect of moisture on respiration of Anguina tritici and A. agrostis. To study the effect of humidity on the respiration of dry A. tritici larvae, the concentration of sodium hydroxide used to absorb carbon dioxide in the neck seal of the diver was changed to produce the desired relative humidity (RH) inside the divers. The following sodium hydroxide concentrations were used to achieve desired RH (Lange, 1956).

RH desired (%)	NaOH concentration (%)
95	5.54
75	18.60
50	28.15
5	saturated with excess of sodium hydroxide

To study changes in respiration during hydration of dry second stage larvae of A. tritici and A. agrostis, galls were opened in sterile distilled water immediately before placement of larvae in divers. The rate of respiration was measured for up to 96 hours in these divers without changing oil or sodium hydroxide seals during this period. Mouth seals were changed when the divers became too heavy after a day or two. To determine the effect of storage of larvae inside the divers, in one set of experiments (with L_2 larvae of A. tritici from 1965 wheat galls) divers were filled each day with fresh nematodes from a supply stored in dishes of water.

Effect of osmotic pressure and composition of the medium. To study the effect of osmotic pressure on respiration, 0.01M, 0.1M, 1M and 2M urea and D-mannitol solutions were used to produce osmotic pressures of 0.224, 2.24, 22.4 and 44.8 atmospheres respectively. Sodium chloride and potassium chloride solutions were used to study the osmotic effect and also the influence of sodium and potassium ions on respiration.

Effect of carbon dioxide on respiration. In order to study the effect of carbon dioxide concentration on respiration, Krebs' (1951) carbon dioxide buffer was used to replace the sodium hydroxide seal in the neck of the Cartesian diver. Care was taken to place these buffer seals away from the bulb of the diver, as the buffer had a tendency to creep into the bulb, and it appeared to be toxic to the nematodes. The carbon dioxide buffer absorbs measurable amounts of oxygen, hence oxygen absorption detected in the blank was deducted from the readings of the experimental divers.

Air-carbon dioxide mixtures used to charge the carbon dioxide buffers and floatation medium were obtained from the Mathesan Company, East Rutherford, New Jersey. These mixtures were not analyzed for purity. The stock carbon dioxide buffer solution and the floatation medium each was aerated with desired air-carbon dioxide mixture for one hour just before the experiment was started using a sintered aerator.

CHAPTER III

RESULTS

Effect of moisture on respiration of second stage larvae of Anguina tritici and A. agrostis. Oxygen consumption could be detected when intact dry wheat galls or bentgrass seed galls with nematode larvae were placed in a Cartesian diver or Warburg flask at 95% RH. Q_{O_2} values of L_2 larvae of A. tritici removed from 1965 wheat galls and kept at different humidities are given in Table 1. In humidities below 95% RH, larvae respired very slowly. During the first six hours after larvae of A. tritici were exposed to 95% RH, the rate of oxygen consumption gradually increased (Table 2).

The initial rate of respiration of A. tritici larvae from old wheat galls was much lower than that of larvae from 1965 galls (Fig. 3). During the 72 hour period after the larvae were placed in water, the highest rate of respiration for larvae from old wheat galls was 2.295 mul/ug dry weight/hour and for larvae from 1965 wheat galls 2.940 mul/ug dry weight/hour.

When the larvae of A. tritici were removed from old galls and placed in water, the rate of respiration gradually rose until 72 hours after hydration and then decreased (Fig. 3). The larvae from the old galls began to move about 60 hours after they were placed in water, whereas the larvae from 1965 wheat galls, when placed in water, became active within four to six hours.

The rate of respiration of larvae from 1965 wheat galls was highest six hours after hydration, when it was measured for the first time,

Fig. 3. Respiratory rates of second stage larvae of Anguina tritici from ten year old and 1965 wheat galls, after removal from galls and placement in water. The rate of respiration of larvae from 1965 galls was measured by 'Open' and 'Closed' systems. (See text). Each point represents a determination of three replications.

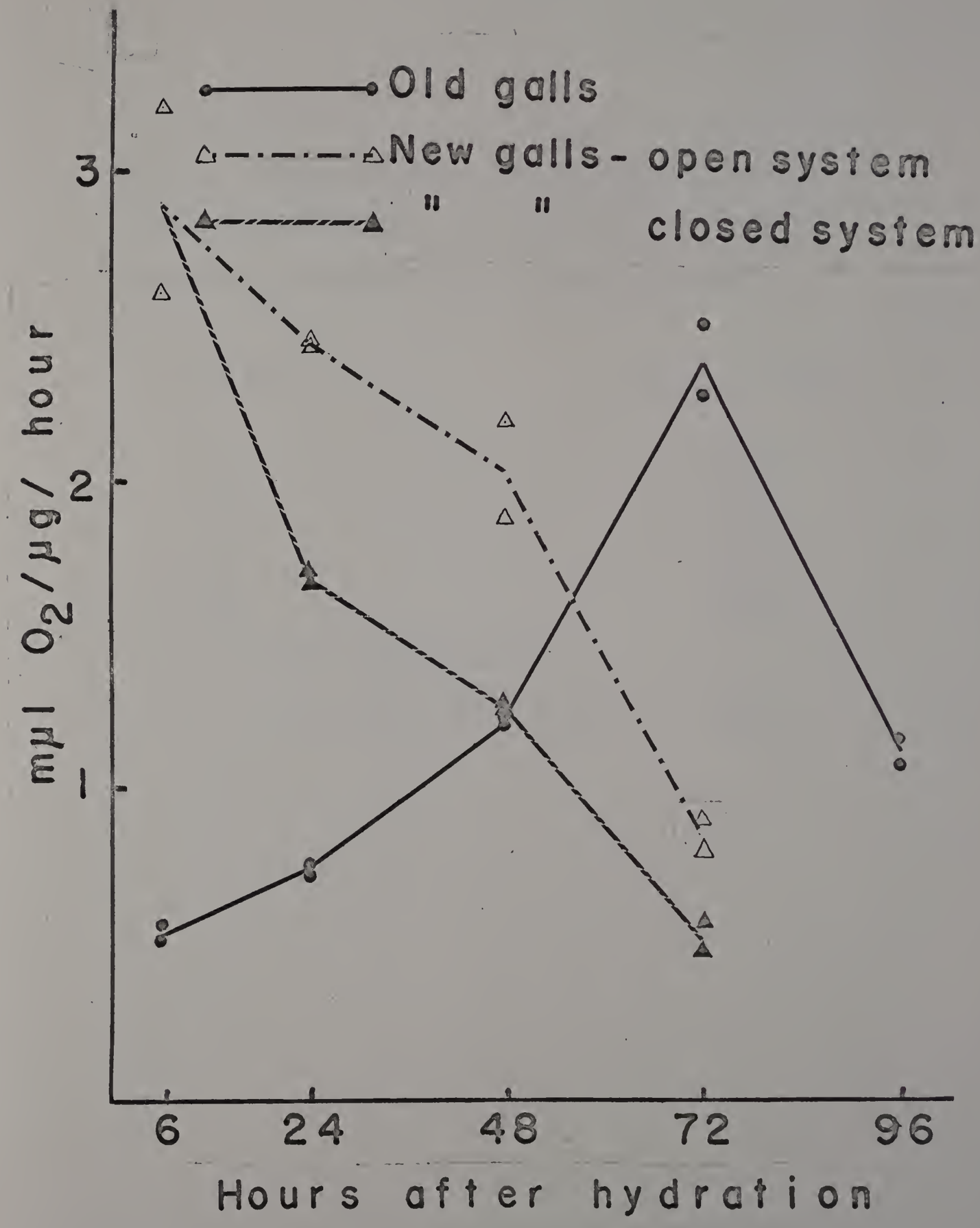

Old galls
New galls - open system
" "
closed system
mμl O2/μg/ hour
3
2
1
6
24
48
72
96
Hours after hydration

TABLE 1. Oxygen consumption of dry larvae of Anguina tritici from 1965 wheat galls during the fifth hour of exposure to different relative humidities at 25°C.

RH (%)	mμl O_2/ μg/ hour
5	0.009
50	0.028
75	0.033
95	1.600
5 (after exposing larvae to 95% humidity for 24 hours)	0.021

TABLE 2. Progressive increase in oxygen consumption of dry L_2 larvae of Anguina tritici removed from 1965 wheat galls in 95% RH.

Hours after exposing larvae to 95% RH	mμl O_2 / μg / hour
3	0.55
4	1.12
5	1.58
6	1.74

TABLE 3. Oxygen consumption of L_2 larvae of Anguina tritici from old wheat galls after placing the dry larvae in water. Each Q_{O_2} value is based on two determinations of three replications each.

Hours after hydration	Q_{O_2}	standard error	Level of statistical significance
6	0.541	± 0.043	n.s.
24	0.745	± 0.014	99.0%
48	1.225	± 0.026	99.9%
72	2.294	± 0.100	99.9%
96	1.136	± 0.058	

TABLE 4. Rate of oxygen consumption of L_2 larvae of Anguina tritici from 1965 wheat galls after placing dry larvae in water, in closed and open systems.

Hours after hydration of larvae	OPEN SYSTEM Q_{O_2}	SE	Level of statistical significance	CLOSED SYSTEM Q_{O_2}	SE	Level of statistical significance	Level of statistical significance between OPEN and CLOSED systems.
6	2.929	± 0.290		2.929	± 0.290		
			n.s.			99%	
24	2.455	± 0.226	95%	1.704	± 0.428		99%
			n.s.			90%	
48	2.050	± 0.164		1.305	± 0.209		98%
			99%			99%	
72	0.873	± 0.183		0.543	± 0.109		n.s.

after which it dropped sharply. Respiratory behavior of this kind was shown when A. tritici larvae were placed in Cartesian divers and respiratory measurements were made for 72 hours without changing the neck seals during this period. Such an arrangement may be called a "Closed System." On the other hand, if each day new larvae were used to load divers, (to provide an "Open System"), a slightly different response was obtained (Fig. 3, Table 4). Under such conditions the respiratory rate did not fall as rapidly.

The rate of respiration of L_2 larvae of A. tritici recovered from 1965 wheat galls decreased sharply during the first few months after harvest and then dropped more gradually (Table 5). It was observed that the time necessary for these larvae to become active after hydration gradually increased during the year after harvest.

Effect of osmotic pressure, ionic composition of medium, nutrients and starvation on respiration. Rates of respiration of L_2 larvae of A. tritici and A. agrostis and adults of D. dipsaci and Pratylenchus penetrans under different osmotic pressures are given in Fig. 4 and Table 6. Data from Wallace and Greet (1964) on Tylenchorhynchus icarus have been included in Figure 4 for comparison. In Figure 4, the rate of respiration in water has been taken as 100 percent and respiratory rates under different osmotic pressures have been expressed as percent of rate of respiration in water. Analyses of variance of Q_{O_2} values under different osmotic pressures have been given in the Appendix.

In the five nematode species under study, with the exception of P.

TABLE 5. Rate of respiration of L_2 larvae of A. tritici from wheat galls from May 1965 harvest six hours after hydration.

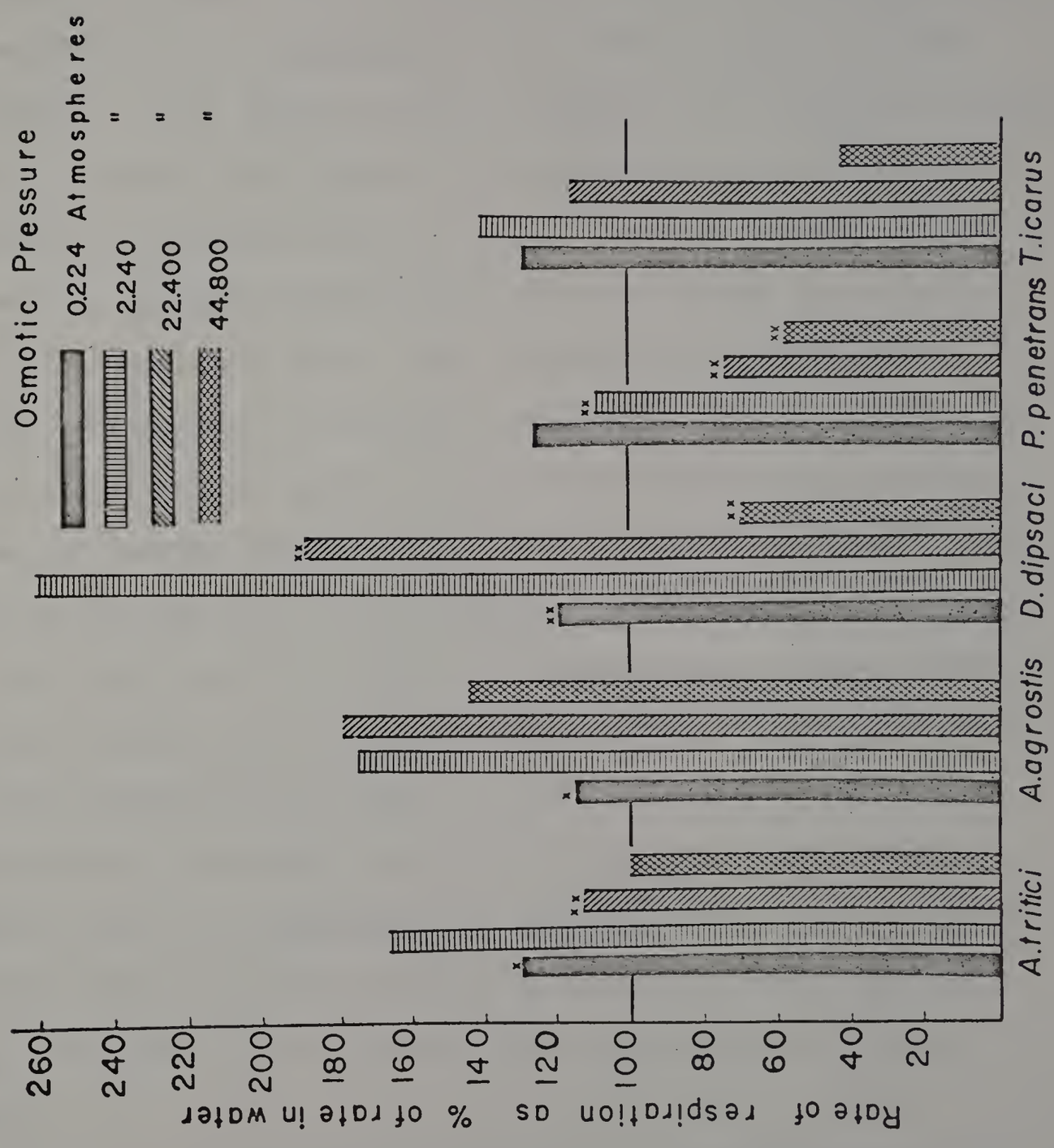
Month of measurement	mμl oxygen/ μg / hour
July, 1965	2.929
August, 1965	1.484
February, 1966	1.221
March, 1966	1.031
July, 1966	0.882

TABLE 6. Influence of increasing osmotic pressure produced by urea, D-Mannitol, potassium chloride and sodium chloride on oxygen consumption of second stage larvae of Anguina tritici and A. agrostis six hours after hydration and adults of Ditylenchus dipsaci and Pratylenchus penetrans.

Chemical used	Osmostic pressure in atmospheres				
	0	0.224	2.24	22.4	44.8
			A. tritici (from 1965 galls)		
Urea	1.031	1.331	1.710	1.168	1.031
D-Mannitol			1.684		
Sodium chloride			1.810	1.429±SE	0.208*
Potassium chloride			1.020	0.390±SE	0.047*
			A. agrostis		
Urea	1.210	1.600	2.112	2.140	1.952
Sodium chloride			2.740	2.840	
			D. dipsaci		
Urea	2.414	2.892	6.309	4.500	1.682
			P. penetrans		
Urea	5.238	6.542	5.690	3.809	3.025
D-Mannitol			5.121		

* The standard error for the respiratory rates of *A. tritici* larvae in 0.5 M sodium chloride and 0.5 M potassium chloride solutions has been shown here. The standard error for the respiratory rates of different species in urea solutions have been given in the Analysis of Variance tables in the Appendix.

Fig. 4. Respiratory rates of second stage larvae of Anguina tritici and A. agrostis and adults of Pratylenchus penetrans and Ditylenchus dipsaci at osmotic pressures of 0.224, 2.24, 22.4 and 44.8 atmospheres produced by urea solutions shown as the percentage of the rate in distilled water. Each percent value is based on two determinations of three to five replications each. Crosses (X) between tops of adjacent bars show that a statistically significant difference exists between the respiratory rates represented by the bars. X = 95% confidence level; XX = 99% confidence level. Percentage values for Tylenchorhynchus icarus have been calculated from the data of Wallace and Greet (1964).


Osmotic Pressure
0.224 Atmospheres
2.240 "
22.400 "
44.800 "
Rate of respiration as % of rate in water
260
240
220
200
180
160
140
120
100
80
60
40
20
A.tritici
A.agrostis
D.dipsaci
P.penetrans
T.icarus

penetrans, the rate of respiration increased as the osmotic pressure of the medium was raised from zero to 2.24 atmospheres. The highest rate of respiration for P. penetrans was at an osmotic pressure of 0.224 atmospheres. In A. tritici larvae, D. dipsaci, and T. icarus the highest rate of respiration was reached at 2.24 atmospheres, and then Q_{O_2} decreased with increasing osmotic pressure. The rate of respiration of L_2 larvae of A. agrostis remained almost the same between 2.24 and 44.8 atmospheres osmotic pressure. When compared to A. agrostis and A. tritici, the decrease in the rate of respiration at 44.8 atmospheres of other species was very marked. A D-mannitol solution used to produce an osmotic pressure of 2.24 atmospheres gave results identical to urea solutions isotonic to it with P. penetrans and L_2 larvae of A. tritici.

The body shape and motility of A. tritici and A. agrostis larvae were not affected when these were placed in water, 10^{-2} M, 10^{-1} M or 1 M urea solutions for ten hours. In 2 M urea solutions larvae were contracted and less motile, but recovered fully when placed back in distilled water. P. penetrans and D. dipsaci became slightly contracted and less motile in 1 M and 2 M urea solutions and took much longer to regain body shape and motility than did A. agrostis and A. tritici larvae.

Sodium chloride solutions (0.05 and 0.5 M) elevated the rate of respiration of L_2 larvae of A. tritici and A. agrostis to the same levels as the isotonic urea solutions. Stimulation of respiration of A. tritici larvae by potassium chloride solutions producing 2.24 and

22.4 atmospheres osmotic pressure was significantly less as compared to stimulation by sodium chloride solutions of same concentration. In potassium chloride solution isotonic to 10^{-1} urea, the rate of respiration was the same as in distilled water. In potassium chloride solution producing 22.4 atmospheres osmotic pressure the rate of respiration was only 27% of the rate in an isotonic sodium chloride solution, a highly significant difference (Table 6).

Three attempts were made to find out if the addition of glucose to the medium would increase the rate of respiration of L_2 larvae of A. tritici from 1965 wheat galls. A single similar attempt was made with adults of A. ritzemabosi. No rise in the rate of respiration of either of these two species after the addition of glucose solution from the side arm of a Warburg flask to make the concentration of glucose in the medium 0.1 mg/ml could be detected.

The respiratory rate of P. penetrans adults dropped sharply for 20 hours after their removal from culture, reaching 50% of the original rate after 15 hours (Fig. 5). Forty-eight hours after removal of nematodes from culture the rate of respiration became nearly constant and there was very little drop in Q_{O_2} from the second day to the tenth day.

The respiratory rate of Tylenchorhynchus claytoni (Steiner, 1937) decreased in a similar manner over a period of five days after removal from culture. The actual rates of respiration obtained from this experiment on T. claytoni have not been given here because nematodes were not available in sufficient number to determine the dry weight of this

species.

Effect of carbon dioxide concentration on respiration. Respiratory rates of A. ritzemabosi, P. penetrans and D. dipsaci at carbon dioxide concentrations of 0, 0.03, 0.1, 0.5, 1.0 and 2.0% are shown in Table 7 and Figure 6. The analyses of variance of these Q_{O_2} values are given in the Appendix.

The rate of respiration of A. ritzemabosi and P. penetrans was higher in 0.03% carbon dioxide as compared to the rate in the total absence of carbon dioxide in the gaseous phase. The rise in the rate of respiration of A. ritzemabosi in the presence of 0.03 percent carbon dioxide was 444% over the rate in 0 percent carbon dioxide. The Q_{O_2} of D. dipsaci decreased as the carbon dioxide concentration was increased from 0 to 0.5%. The fall in the rate of respiration was significant between 0 and 0.03% carbon dioxide, but not between 0.03 and 0.5% carbon dioxide concentration. When carbon dioxide concentration was raised from 0.03 to 0.1%, the respiratory rate dropped significantly in P. penetrans and A. ritzemabosi. The rate of respiration of A. ritzemabosi rose very slightly when the carbon dioxide concentration in the gas phase was raised from 0.1 to 1.0 percent, while the same increase resulted in a greater rise of Q_{O_2} of P. penetrans. The respiratory rate of D. dipsaci rose more than six times when the carbon dioxide concentration was increased from 0.5 to 1.0%.

An increase in carbon dioxide concentration from 1 to 2% lowered the rate of respiration of A. ritzemabosi and D. dipsaci. The decrease

Fig. 5. The effect of storage in distilled water on the rate of respiration of Pratylenchus penetrans. Each point represents a determination of two to three replicates.

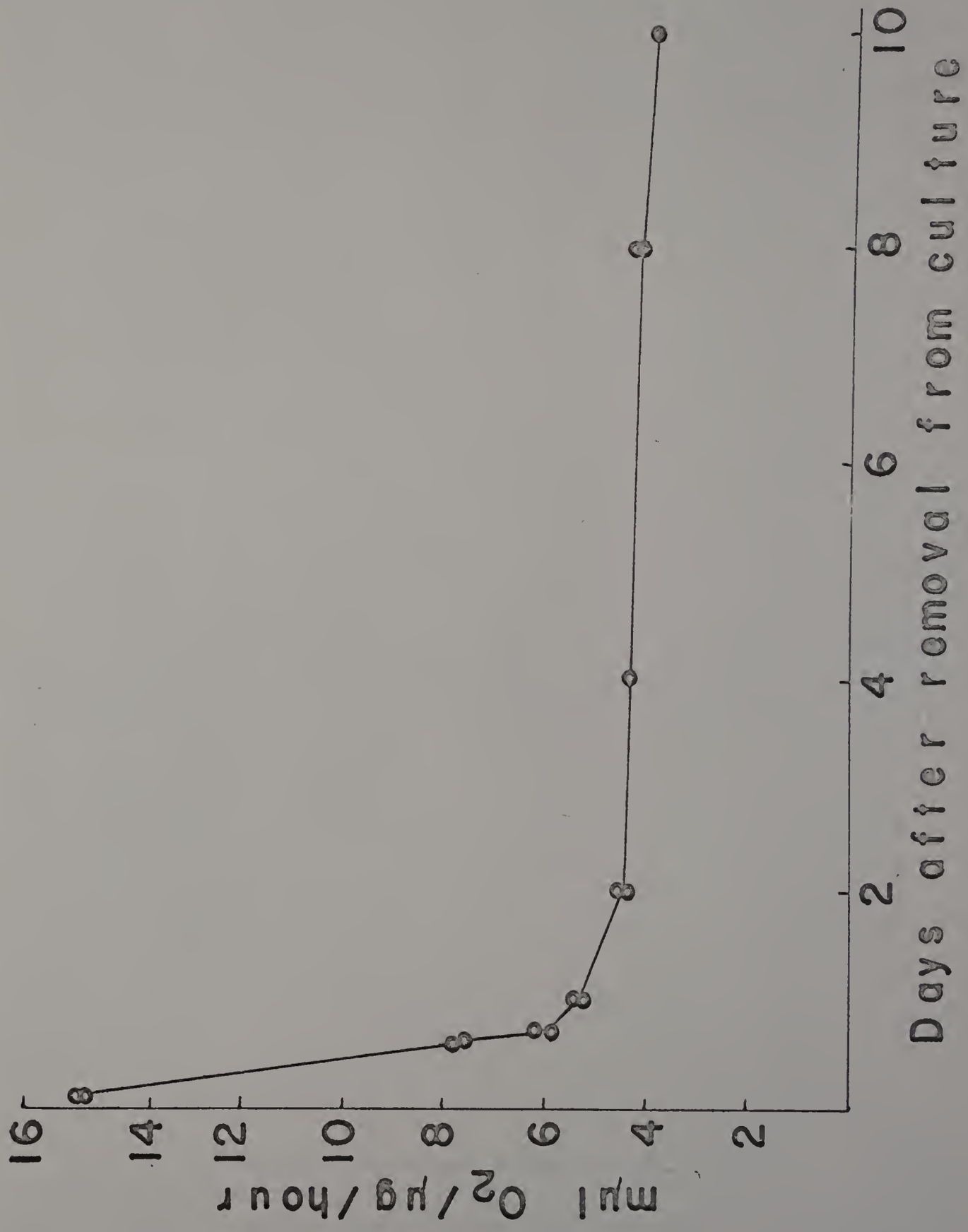

mµl O_2/µg/hour
16
14
12
10
8
6
4
2
2
4
6
8
10
Days after removal from culture

Fig. 6. The influence of different carbon dioxide concentrations on the respiratory rates of Pratylenchus penetrans, Aphelenchoides ritzemabosi and Ditylenchus dipsaci. Each point represents a determination of four to five replications.

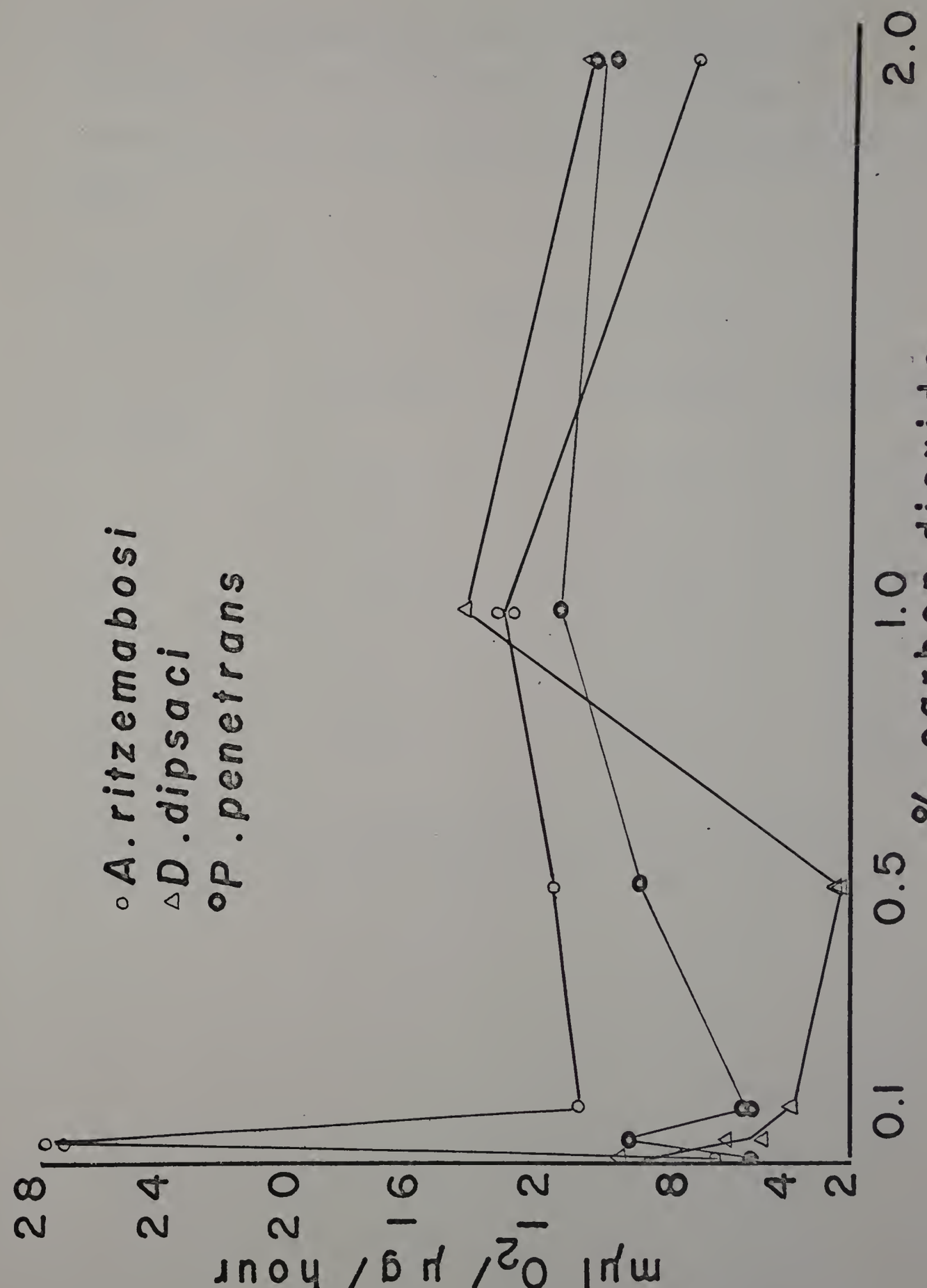

mul O_2/ mg/ hour
28
24
20
16
12
8
4
2
0.1
0.5
1.0
2.0
% carbon dioxide
○A. ritzemabosi
△D. dipsaci
●P. penetrans

TABLE 7. Respiratory rates of Ditylenchus dipsaci, Aphelenchoides ritzemabosi and Pratylenchus penetrans in carbon dioxide concentrations -- 0, 0.03, 0.1, 0.5, 1.0 and 2.0 percent in the gas phase.

Carbon dioxide Concentration (percent of gas phase)	μl oxygen/ μg/ hour		
	D. dipsaci	P. penetrans	A. ritzemabosi
0	9.380 a*	5.239 a*	6.114 a*
0.03	4.618 b	8.995 b	27.235 b
0.10	3.684 bc	4.874 a	10.672 c
0.50	2.308 c	8.647 b	11.416 c
1.00	14.317 d	11.190 b	13.332 c
2.00	10.360 a	10.221 b	6.896 a

* Q_{O_2} values for each nematode species followed by the same letter are not significant at 95 percent confidence level.

in Q_{O_2} of P. penetrans as carbon dioxide concentration increased from 1 to 2% was not statistically significant.

All three species were actively moving after eight hours exposure to carbon dioxide concentrations from 0.03 to 2.0 percent. In 1 or 2% carbon dioxide, D. dipsaci appeared to be more active than at lower concentrations.

Effect of temperature on the rate of respiration. The rates of respiration of adults of P. penetrans, D. dipsaci and A. ritzemabosi and L_2 larvae of A. tritici at temperatures 10, 15, 22, 30 and 35°C are shown in Table 8 and Figure 7. Respiration of D. dipsaci was also measured at 5°C.

The rate of respiration of all the species studied except for D. dipsaci increased as the temperature was raised from 10° to 35°C. The rate of respiration of D. dipsaci decreased sharply as the temperature was raised above 22°C. The rate of respiration of D. dipsaci remained high even at 5°C. The respiratory rate of other species could not be determined at 5°C. The rate of respiration of these species was quite low at 10°C. Taking into consideration the difficulties in operating the Cartesian diver respirometer at 5°C, it was not considered worthwhile to pursue respiratory measurements at this temperature. The rate of respiration of A. tritici larvae gradually increased with a rise in temperature from 10° to 35°C. The increase in the respiratory rate of P. penetrans with the increase in temperature was almost linear.

Fig. 7. Respiratory rates of Pratylenchus penetrans, Ditylenchus dipsaci and Aphelenchoides ritzemabosi adults and second stage larvae of Anguina tritici from 1965 wheat galls at temperatures -- 5, 10, 15, 22, 30 and 35°C. Each point represents a determination of four to five replicates.

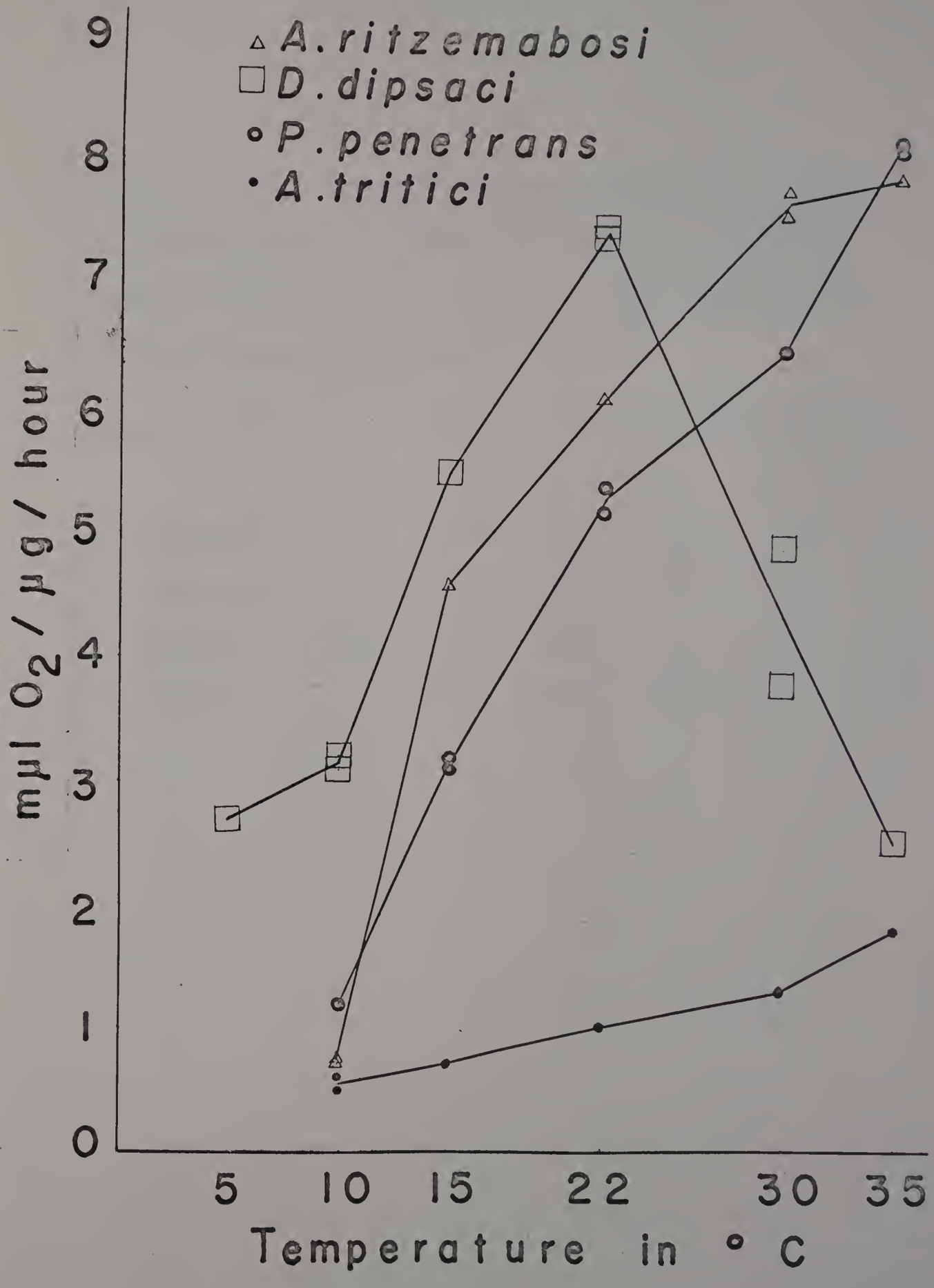

A. ritzemabosi
D. dipsaci
P. penetrans
A. tritici
mμl O2 / μg / hour
0
1
2
3
4
5
6
7
8
9
5
10
15
22
30
35
Temperature in ° C

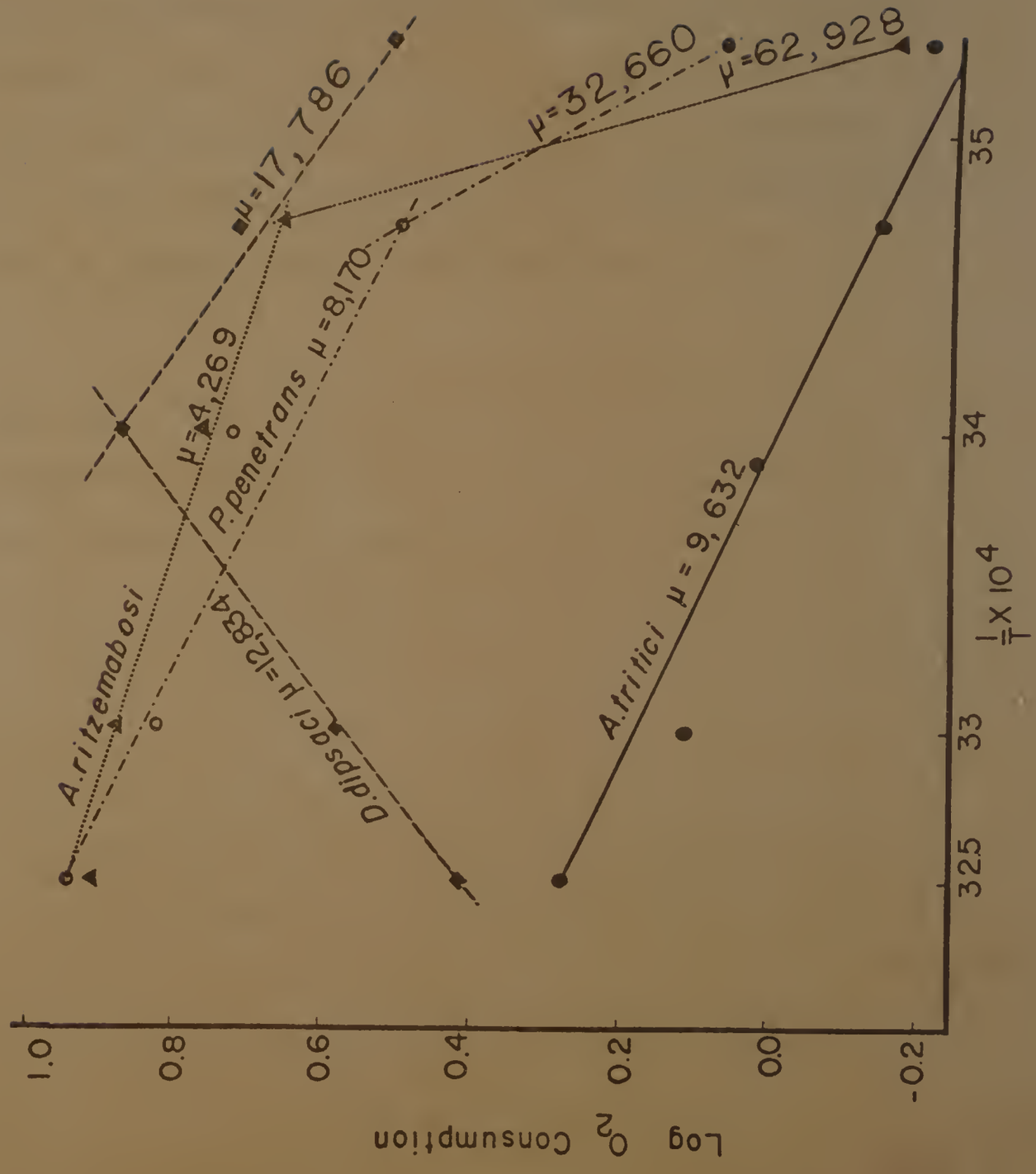
TABLE 8. Respiratory rates of Aphelenchoides ritzemabosi, Ditylenchus dipsaci and Pratylenchus penetrans adults and second stage larvae of Anguina tritici (from 1965 galls) at temperatures -- 5, 10, 15, 22, 30 and 35°C.

Nematode species	Rate of respiration at temperature(°C)					
	5	10	15	22	30	35
A. ritzemabosi		0.696	4.606	6.121	7.452	7.969
P. penetrans		1.195	3.174	5.239	6.543	8.652
D. dipsaci	2.793	3.247	5.536	7.433	3.804	2.567
A. tritici		0.600	0.705	1.031	1.294	1.863

TABLE 9. Q_{10} values for Aphelenchoides ritzemabosi, Ditylenchus dipsaci and Pratylenchus penetrans adults and Anguina tritici larvae in the temperature ranges 10 - 20°C and 25 - 35°C.

Nematode species	Temperature range	Q_{10}
A. tritici	10 - 20	1.6
	25 - 35	1.6
A. ritzemabosi	10 - 20	7.6
	25 - 35	1.4
P. penetrans	10 - 20	3.9
	25 - 35	1.4
D. dipsaci	10 - 20	2.2

Fig. 8. Oxygen consumption of adults of Pratylenchus penetrans, Ditylenchus dipsaci and Aphelenchoides ritzemabosi and second stage larvae of Anguina tritici expressed according to Arrhenius formula. Data are taken from Table 8.


Log O_2 Consumption
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
325
33
34
35
$\frac{1}{T} \times 10^4$
A.ritzemabosi
μ=17,786
μ=4,269
P.penetrans μ=8,170
D.dipsaci μ=12,884
A.tritici μ=9,632
μ=32,660
μ=62,928

The Q_{10} values in the temperature ranges 10°-20°C and 25°-35°C have been given in Table 9. The rates of respiration have been plotted according to the Arrhenius equation in Figure 8, which also includes the thermal increment values (μ).

All the species were active and motile in the temperature range of 10° to 35°C except A. ritzemabosi, which became coiled and inactive at 35°C. D. dipsaci was quite active at 5°C.

CHAPTER IV

DISCUSSION

Respiratory rates of those plant-parasitic nematodes studied fell within the range of Q_{O_2} values known for nematodes parasitic in animals. When comparing Q_{O_2} values of nematodes from these two different habitats, however, it must be kept in mind that respiratory rates for animal-parasitic nematodes have been usually measured at 37°C and the rate of plant-parasitic nematodes were determined in the present studies at 22°C.

Osmotic pressure, carbon dioxide, moisture and temperature are important factors in the environment of plant-parasitic nematodes. These environmental factors fluctuate widely in soil as well as inside host plants. Osmotic pressure inside tomato roots varies from five to 16 atmospheres (Spector, 1956). Osmotic pressure and carbon dioxide concentration in soil may change sharply after a rain or as a result of drought. Plant-parasitic nematodes are continually exposed to wide limits of temperature, carbon dioxide concentration, osmotic pressure, and moisture--much more so than are animal-parasitic nematodes. It is probable that each nematode species has developed its own tolerances to different degrees of environmental stress, according to its habit, habitat and geographical distribution.

Plant-parasitic nematodes pass at least part of their life cycles in soil, where they live in water films surrounding soil pore spaces (Wallace, 1963). Carbon dioxide produced as a result of respiration diffuses out of water films slowly. Moreover, carbon dioxide is readily

soluble in water, resulting in a high concentration of carbon dioxide in the environment of nematodes in soil. The high concentration of carbon dioxide in soil may explain the profound influence of carbon dioxide on nematode respiration. Respiration of plant-parasitic nematodes is inhibited in the absence of carbon dioxide from their environment. The rate of oxygen consumption of plant-parasitic nematodes tends to decrease after about five hours storage inside Cartesian divers with a potassium hydroxide neck seal (Bhatt and Rohde, 1965). A relatively low rate of respiration of A. tritici larvae, when measured by using the same divers without changing the neck seals during a 72-hour period (Fig. 3; Table 4--closed system) may be due to total absence of carbon dioxide from inside the divers for long periods. A similar inhibitory effect in the absence of carbon dioxide on the respiration of P. penetrans has been reported by Rohde (1960). Such a decline in the respiratory rate cannot be explained on the basis of a drop in oxygen tension within the respiration vessel. In soil nematodes a 10% decrease in oxygen tension does not influence the rate of oxygen consumption (Nielsen, 1949, 1961). In the present studies the oxygen tension inside the Cartesian divers did not fall more than 10% in six hours. The oxygen tension in divers with carbon dioxide buffer was even lower, and yet nematodes in these divers respired at a much higher rate. It is suggested that absence of carbon dioxide inside divers for long periods is responsible for slowing of the rate of respiration of nematodes.

P. penetrans passes most of its life cycle in soil. These nematodes cause lesions a few cells deep in roots. The lesions soon break down and the nematodes come out in soil again. Rhizosphere microorganisms are active so that the carbon dioxide concentration in the environment of these nematodes is high, and they appear to be stimulated by these higher concentrations. On the other hand, respiration of A. ritzemabosi is highest in air. This might be an adaptation to its life in leaves and other above-ground parts of plants, where carbon dioxide concentrations are closer to that of the atmosphere.

Adults of P. penetrans and D. dipsaci and second stage larvae of A. tritici and A. agrostis respire within a range of osmotic pressure from 0 to 44.8 atmospheres. P. penetrans, which may be considered a typical plant-parasitic nematode, respires maximally at 0.224 atmospheres osmotic pressure, which is close to the range of optimum osmotic pressure (0.87% sodium chloride) for some of the animal-parasitic nematodes (von Brand, 1943; Davey, 1938). The tolerance range of P. penetrans is wide on either side of 0.224 atmospheres. Even at 2.24 atmospheres, its rate of respiration is higher than in distilled water. Since the respiratory rate of plant-parasitic nematodes is high over a wide range of osmotic stress, there are indications that these nematodes possess some osmoregulatory mechanism (Potts and Parry, 1964). The presence of such a mechanism in Heterodera and Meloidogyne was suggested by Dropkin (1955).

Second stage larvae of A. tritici and A. agrostis and preadults

of D. dipsaci are well adapted to resist drying and accompanying high osmotic pressures. These nematodes seem to have a capacity to expend energy to pump in water lost due to exosmosis. The increased respiration of these nematodes in 2.24 to 44.8 atmospheres osmotic pressure may reflect this extra work. The rate of respiration of A. tritici and A. agrostis larvae does not drop significantly, even at 22.4 and 44.8 atmospheres osmotic pressure, which may be related to their capacity to survive a high degree of desiccation for a long time. The fall in the respiratory rate of A. agrostis larvae when the osmotic pressure is raised from 2.24 to 22.4 and 44.8 atmospheres is not significant, whereas the rate of respiration of A. tritici larvae drops significantly when the osmotic pressure of the medium is raised from 2.24 to 22.4 atmospheres. This difference in the response of A. tritici and A. agrostis larvae to increasing osmotic pressure might be explained by the difference in the structure of the galls which enclose the larvae. The wheat gall has a round to oval cavity with thick walls, which may protect the nematode larvae from the drying effect of the environment. The bentgrass seed galls, on the other hand, are elongated and brittle with very thin walls and do not afford as good protection.

Although preadults of D. dipsaci are the stage most resistant to desiccation, adults also exhibit this property to a limited degree. D. dipsaci and Tylenchorhynchus icarus resemble A. tritici larvae in that their maximum respiration is at 2.24 atmospheres osmotic pressure,

but differ in that their respiratory rate falls at 44.8 atmospheres in a manner similar to that of P. penetrans. This may indicate that D. dipsaci and T. icarus are not as well adapted to survive desiccation as are the larvae of A. tritici and A. agrostis.

D. dipsaci spends most of its life closely associated with the host plant, in the storage tissue such as bulbs. Tolerance to high osmotic pressure and high carbon dioxide concentrations may be of survival value to D. dipsaci inside the bulbs. When the bulbs containing D. dipsaci rot, osmotic pressure would be high due to breakdown of starch to sugar. Cellulose, pectins, starch and chitin of the plant are broken down by different hydrolytic enzymes secreted by D. dipsaci, thus increasing the osmotic pressure still further inside these storage organs.

The effect of osmotic pressure on respiration has been studied using mainly urea. The influence of urea on respiration may be due not only to osmotic pressure, but its toxic nature may also be responsible to some extent in influencing respiration of nematodes. D-Mannitol and sodium chloride solutions stimulated respiration in a way similar to urea.

At the stage when the surrounding medium as well as the tissues of the nematodes are dried up, osmotic stress is probably no longer a problem for drought resistant nematodes as there seems to be no free water left in the galls or the nematodes. At such an advanced stage of desiccation the nematodes are in a state of anhydrobiosis. The

respiration of intact galls with A. tritici or A. agrostis larvae is measurable, but it is not possible to say how much oxygen intake is by plant material of the galls. Larvae of A. tritici removed from 1965 wheat galls have been found to respire in relative humidities as low as 5%. The metabolic activity of L_2 larvae of A. tritici declines during the first year inside wheat galls (Table 5). It is difficult to say how much each of several factors--aging, starvation and gradual dehydration of nematode tissues as well as of the plant tissue of the galls contributes to the decline of Q_{O_2} of larvae stored inside galls. Low oxygen consumption of Anguina larvae would conserve reserve food matter, which may be required when the larvae emerge from the galls, and increase survival.

The increase in rate of respiration of A. tritici larvae with increase in relative humidity from five to 95 percent is not logarithmic, as has been reported for the drought resistant tardigrade, Macrobiotus hufelandi (Pigon and Weglarska, 1955). Rate of respiration of A. tritici larvae remains very low in humidities below 95% RH. When desiccated larvae of A. tritici are exposed to a given humidity there is a steady rise in the rate of respiration (Table 2). This might be because, as the moisture penetrates the mass of larvae, more and more larvae begin to respire, or because the metabolic rate of individual larvae increases as tissues absorb more water.

When wheat galls with A. tritici larvae are planted with wheat seeds in order to obtain experimental infection of wheat, a very high

and constant humidity is required to achieve infection. Reduction in moisture content may inhibit nematode activity by producing thin films in which nematodes cannot move (Wallace, 1963).

A. tritici larvae from old galls take longer (72 hours) to reach the level of highest metabolic activity than do larvae from 1965 wheat galls (approximately six hours). This might be because the former are more dehydrated during storage inside the wheat galls. Another factor contributing to initially high rate of respiration after hydration of larvae could be an oxygen debt, which might be expected under storage conditions of the desiccated larvae in large numbers inside galls.

An initial rise in the rate of respiration of A. tritici larvae after hydration does not seem to be correlated with larvae becoming motile. Although the highest rate of respiration after hydration of larvae coincides with the time when larvae reach maximum motility, the larvae continue to be motile after the rate of respiration begins to fall again. The investigations of Nielsen (1949) and Santmeyer (1956) indicate that the body movements of free living nematodes require very little energy.

A fall in the rate of respiration of A. tritici larvae, after an initial rise, may be due to either starvation or aging, whose effects on Q_{O_2} are difficult to separate (van Gundy, 1965). Similarly, aging and starvation may be responsible for the fall in the respiration rate of P. penetrans and T. claytoni stored in distilled water. Fall

in the rate of respiration of P. penetrans stored in distilled water after removal from culture follows the pattern obtained by Rohde (1960) for this nematode after removal from soil. The rate of respiration of some free-living soil nematodes (Dorylaimus obtusicaudatus, Mononchus papillatus, Plectus granulosus, Pontonema vulgare) however, has been reported to remain stationary over a seven day period after removal from soil (Nielsen, 1949).

Stimulation of respiration of adults of A. ritzemabosi and second stage larvae of A. tritici by addition of glucose to the medium could not be detected. It is possible that these nematodes are not able to utilize exogenous glucose in the absence of a suitable feeding stimulus, as is required by some animal-parasitic nematodes (Warren, et al., 1962; Fernando and Wong, 1964; Roberts and Fairbairn, 1965). Plant-parasitic nematodes normally feed by piercing cell walls with the stylet and perhaps liquid media are not ingested.

Potassium ions do not stimulate respiration of L_2 larvae of A. tritici as do sodium ions. A 0.5 M potassium chloride solution inhibits respiration. In this respect A. tritici larvae resemble Litomosoides carinii (Bueding, 1949) and differ from the larvae of Eustrongyloides ignotus (von Brand, 1943).

The rate of most biological processes, including respiration, increases with an increase in temperature, reaching a maximum, beyond which the rate of activity tends to decline. The temperature-respiratory rate curves (Fig. 7) for several plant-parasitic nematodes show

that the optimum temperature for respiration varies with the species. This optimum for respiration appears to be related to optimum temperatures for other activities such as motility, infectivity, and reproduction of different nematode species.

The optimum temperature range for mobility and reproduction of D. dipsaci is 15 to 20°C (Seinhorst, 1950; Wallace, 1958, 1961; Barker, 1959), which coincides with the temperature at which the rate of respiration is maximum. D. dipsaci, which is found mainly in cool climates, is known to survive well at low temperatures. When alfalfa crowns infested with D. dipsaci were exposed throughout the winter in the mountains of Utah, where night temperatures ranged from -20 to -30° C for a period of four months, the nematodes were not injured (Thorne, 1961). Adaptability of this nematode to withstand low temperature is shown in its high rate of respiration at 5°C.

The highest rate of respiration of P. penetrans measured between 10 and 35°C occurred at 35°C, which coincides with the optimum soil temperature for activity of the related species P. minyus (Mountain, 1954). A. ritzemabosi is a characteristic nematode of temperate climates (Winslow, 1960) and its highest rate of respiration measured between 10 and 35°C is in the range of 30 to 35°C. Optimum temperature range for the activity of A. tritici larvae is not known, although this species is distributed mainly in warm climates. The temperature-respiratory rate curve for the larvae indicates this range should be in the neighborhood of 30°C.

Crozier (1926a, 1926b) reported that temperature characteristic values change generally at 4.5, 9, 15, 20, 27, and 30°C, the most frequent break being at 15°C. Change in the μ value of P. penetrans and A. ritzemabosi at 15°C coincides with the temperature below which the activity of these nematodes is markedly reduced (Fig. 8). A sharp change in the μ value of D. dipsaci at 22°C reflects adverse conditions above this temperature.

Temperature not only has a direct bearing on the rate of biological processes, but influences other environmental factors as well. Solubility of carbon dioxide in water decreases with an increase in temperature. At 30°C, the amount of carbon dioxide dissolved in water is only three-fifths of the amount soluble at 10°C. On the other hand, the carbon dioxide content in soil may increase at higher temperatures because of higher microbial activity. In soil, higher temperatures may result in a long term rise in osmotic pressure, due to increased evaporation of water. Osmotic pressure inside plants tends to drop slightly with a rise in temperature.

Green parts of plants exposed to sunlight would provide a stimulatory environment for Aphelenchoides ritzemabosi. High temperatures accompanying sunlight elevate the respiratory rate of this nematode. At such a high temperature solubility of carbon dioxide in cell sap will be low and photosynthesis would further remove carbon dioxide, which again is stimulatory to the metabolic rate of A. ritzemabosi. The drought resistant nature of A. ritzemabosi indicates that it should

be able to tolerate the high osmotic pressure due to photosynthetic activity inside the foliar parts of plants.

Pratylenchus penetrans, on the other hand, appears to be well adjusted to life in soil, where it can tolerate well high concentration of carbon dioxide due to decay and microbial activity and high osmotic pressure accompanying high temperature.

Ditylenchus dipsaci is well adapted to survive inside storage organs of plants, where carbon dioxide concentration and osmotic pressure are expected to be high, particularly when these organs begin to rot. Storage organs of plants can survive well at low temperatures as compared to growing parts. D. dipsaci inside the overwintering storage organs are able to tolerate low temperatures.

Second stage larvae of Anguina tritici inside developing wheat galls should have high metabolic activity, as the high temperatures accompanied by high osmotic pressures would stimulate respiration of this nematode. After gall formation these larvae go into a state of anhydrobiosis.

CHAPTER V

SUMMARY

Respiratory rates of those plant-parasitic nematodes studied fell within the range of Q_{O_2} values known for animal-parasitic nematodes in the same size range.

The different species appeared to have different degrees of tolerance to extremes of temperature, humidity, osmotic pressure, and carbon dioxide concentration. Much of the life cycle of plant-parasitic nematodes is spent in the soil where these factors are highly variable. In some cases tolerance would seem to enable nematodes to survive better in their particular habitat.

Carbon dioxide accumulates in the soil and the stimulation of respiration by one or two percent carbon dioxide would make this a favorable environment. Those species which are normally found in soil are stimulated by these levels whereas A. ritzemabosi, a foliar form, is stimulated most by the carbon dioxide level found in air. The absence of carbon dioxide appears to be the best explanation for the fall in the respiration rate of nematodes stored in Cartesian divers containing NaOH.

Adults of D. dipsaci and P. penetrans and second stage larvae of A. tritici and A. agrostis respire within a range of osmotic pressures from 0 to 44.8 atmospheres. They are more tolerant of extremes of osmotic stress than are animal parasites, perhaps because their environment is more variable. Respiration of drought-resistant stages is stim-

ulated by increasing osmotic pressure which, in nature, accompanies drought. This may reflect extra work done by the nematodes to pump in water to replace that which is lost. D. dipsaci specimens that occur in bulbs and other storage tissues would be exposed to high osmotic pressures as these tissues break down. Breakdown would be accompanied by high carbon dioxide levels as well.

When the surrounding medium dries up, nematode tissues contain very little water and the animals themselves either die or enter a state of anhydrobiosis. The metabolic rate of dry larvae of A. tritici and A. agrostis is extremely low and consumption of food reserves is probably negligible, enabling them to exist for years in this state. Once these larvae are removed from galls, however, humidity levels as low as 5% RH will stimulate respiration, and 95% RH results in a dramatic return to high oxygen consumption as well as bodily movement.

Nematodes stored in distilled water were found to respire at gradually lower rates, probably because of starvation. The addition of glucose did not stimulate respiration either because it is not utilized by nematodes or, more likely, because it is not taken up under these conditions. The addition of sodium ions stimulated respiration of A. tritici larvae, whereas potassium ions did not.

Optimum temperatures for respiration of the different species corresponded generally to optimum temperatures for other activities of the same species. Ditylenchus dipsaci occurs in northern climates, reproduces best at low temperatures, A. ritzemabosi occurs in temperate

climates, and A. tritici and P. penetrans reproduce best at high temperatures. Optimum temperatures for respiration of D. dipsaci is 20°C, for A. ritzemabosi is 25°C and for A. tritici and P. penetrans is 30° C.

LITERATURE CITED

Barker, K. R. 1959. Studies on the biology of stem nematode. Phytopathology 49: 315.

Bhatt, B. D. and Rohde, R. A. 1965. Respiratory behavior of some nematodes of the family Tylenchidae. (Abstr.) Phytopathology 55: 1283.

Blake, C. D. 1961. Importance of osmotic potential as a component of the total potential of the soil water on the movement of nematodes. Nature 192: 144-145.

Bloom, J. R. 1963. Effect of temperature extremes on the wheat seed gall nematode, Anguina tritici. Plant Disease Reporter 47: 938-940.

Bosher, J. E. and McKeen, W.E. 1954. Lyophilization and low temperature studies with the bulb and stem nematode Ditylenchus dipsaci (Kuhn, 1858) Filipjev. Proc. Helm. Soc. Wash. 21:113-117.

Bueding, E. 1949. Studies on the metabolism of the filarial worm, Litomosoides carinii. J. Exp. Med. 89:107-130.

_____. 1962. Comparative aspects of carbohydrate metabolism. Fed. Proc. 21:1039-1046.

Crozier, W.J. 1926a. Note on the distribution of critical temperatures for biological processes. J. Gen. Physiol. 9:525-529.

_____. 1926b. The distribution of temperature characteristics for biological processes. Critical increments for heart rates. J. Gen. Physiol. 9:531-546.

Davey, D.G. 1938. Studies on the physiology of the nematodes of the alimentary canal of sheep. Parasitology 30:278-295.

Dropkin, V.H. 1955. The relations between nematodes and plants. Exptl. Parasitol. 4:282-322.

Fairbairn, D. 1954. The metabolism of Heterakis gallinae. II. Carbon dioxide fixation. Exptl. Parasitol. 3:52-63.

Fairbairn, D. 1957. The Biochemistry of Ascaris. Exptl. Parasitol. 6:491-554.

Feder, W.A. 1960. Osmotic destruction of plant parasitic and saprophytic nematodes by the addition of sugars to soil. Plant Dis. Reporter 44:883-885.

Feder, W.A. and Feldmesser, J. 1955. Further studies on plant parasitic nematodes maintained in altered oxygen tension. J. Parasitol. 41:47.

Feldmesser, J. and Feder, W.A. 1954. Some effects of altered oxygen tensions on certain plant parasitic and soil inhabiting nematodes in vitro. J. Parasitol. 40:18.

Fernando, M.A. and Wong, H.A. 1964. Metabolism of hookworms. II. Glucose metabolism and glycogen synthesis in adult female A. caninum. Exptl. Parasitol. 15:284-292.

Fielding, M.J. 1951. Observations on the length of dormancy in certain plant infecting nematodes. Proc. helm. Soc. Wash. 18: 110-112.

Giese, A.C. 1962. Cell Physiology. W. B. Saunders Co., Philadelphia. 534 p.

Holter, H. 1943. Technique of the cartesian diver. Compt. Rend. Trav. Lab. Carlsberg. Ser. Chim. 24:399-478.

Kampfe, L. 1959. Über Möglichkeiten der Physiologisch- ökologischen Arbeitsweise in der Nematodenforschung. Verh. d. Deutsch. Zool. 25:378-386.

Keilin, D. 1959. The problem of anabiosis or latent life: history and current concept. Proc. Roy. Soc. (London) 150:149-191.

Klingler, J. 1965. On the orientation of plant nematodes and of some other soil animals. Nematologica 11:4-18.

Krebs, H.A. 1951. The use of 'CO_2 buffers' in manometric measurements of cell metabolism. Biochem. J. 48:349-359.

Krogh, A. 1916. The respiratory exchange of animals and man. Longmans, Green and Co. London. 276 p.

Krusberg, L.R. 1959. Investigations on the life cycle, reproduction, feeding, habits and host range of Tylenchorhynchus claytoni. Steiner. Nematologica 4:187-197.

_____. 1961. Studies on the culturing and parasitism of plant parasitic nematodes, in particular Ditylenchus dipsaci and Aphelenchoides ritzemabosi on alfalfa tissue. Nematologica 6: 181-200.

Lange, N.R. 1956. Handbook of Chemistry. Handbook Publishers, Inc., Sandusky. 1969p.

Laser, H. 1942. A critical analysis of the tissue slices method in manometric experiments. Biochem. J. 36:319-335.

Linderstrøm=Lang, K. 1943. On the theory of the cartesian diver micro-respirometer. Compt. rend. trav. lab. Carlsberg, Ser. Chim. 24:334-398.

Lee, D.L. 1961. Two new species of cryptobiotic (anabiotic) fresh-water nematodes, Actinolaimus hintoni sp. nov. and Dorylaimus keilini sp. nov. (Dorylaimidae). Parasitology 51:237-240.

_____. 1965. The physiology of nematodes. W.H. Freeman and Co., San Francisco. 154 p.

Loomis, W.F. 1957. Sexual differentiation in Hydra. Control by carbon dioxide tension. Science 126:735-739.

Machmer, J.H. 1958. Effect of soil salinity on nematodes in citrus and papaya plantings. J. Rio Grande Valley hort. Soc. 12:57-60.

Mountain, W.B. 1954. Studies of nematodes in relation to brown root rot of tobacco in Ontario. Can. J. Bot. 32:737-759.

_____. 1955. A method of culturing plant parasitic nematodes under sterile conditions. Proc. helm. Soc. Wash. 22:49-52.

Myers, R.F. and Krusberg, L.R. 1965. Organic substances discharged by plant parasitic nematodes. Phytopathology 55:429-437.

Nielsen, C.O. 1949. Studies on the soil microfauna. II. The soil inhabiting nematodes. Natura Jutlandica 2:1-131.

_____. 1961. Respiratory metabolism of some populations of enchytraeid worms and free living nematodes. Oikos 12:17-35.

Pardee, A.B. 1949. Measurement of oxygen uptake under controlled pressures of carbon dioxide. J. Biol. Chem. 179:1085.

Passey, R.F. and Fairbairn, D. 1955. The respiration of Ascaris lumbricoides eggs. Can. J. Biochem. Physiol. 33:1033-1046.

Peacock, F.C. 1957. Studies on root knot nematodes of the genus Meloidogyne in the Gold Coast. Part 2. Nematologica 2:114-122.

Pigon, A. and Weglarska, B. 1955. Rate of metabolism in tardigrades during active life and anabiosis. Nature 176:121.

Potts, W.T.W. and Parry, G. 1964. Osmotic and ionic regulation in animals. The Macmillan Co., New York. 423 p.

Roberts, L.S. 1961. The influence of population density on patterns and physiology of growth in Hymenolepis diminuta (Cestoda: Cyclophyllidea) in the definitive host. Exptl. Parasitol. 11: 332-337.

Roberts, L.S. and Fairbairn, D. 1965. Metabolic studies on adult Nippostrongylus brasiliensis (Nematoda: Trichostrongyloidea). J. Parasitol. 51:129-138.

Rogers, W.P. 1948. The respiratory metabolism of parasitic nematodes. Parasitology 39:105-109.

_____. 1949a. On the relative importance of aerobic metabolism in small nematode parasites of the intestinal tract. I. Oxygen tensions in the normal environment of the parasites. Austrl. J. Sci. Res. B. 2:157-165.

_____. 1949b. On the relative importance of aerobic metabolism in small nematode parasites of the intestinal tract. II. The utilization of oxygen at low partial pressures by small nematode parasites of the alimentary tract. Austrl. J. Sci. Res. B. 2: 166-174.

_____. 1960. The physiology of inactive processes of nematode parasites. The stimulus from the animal host. Proc. Roy. Soc. B. 152:367-386.

Rohde, R.A. 1960. The influence of carbon dioxide on respiration of certain plant parasitic nematodes. Proc. helm. Soc. Wash. 27: 160-164.

Root, W.S. 1930. The influence of carbon dioxide upon the oxygen consumption of Paramecium and the egg of Arbacia. Biol. Bull. 59:48-62.

Russell, E.J. 1950. Soil conditions and plant growth. 8th edition. Longmans, Green and Co., London. 635 p.

Santmeyer, P.H. 1956. Studies on the metabolism of Panagrellus redivivus (Nematoda: Cephalobidae). Proc. helm. Soc. Wash. 23:30-36.

Saz, H.J. and Hubbard, J.A. 1957. The oxidative decarboxylation of malate by Ascaris lumbricoides. J. Biochem. 225:921-933.

Saz, H.J. and Vidrine, A. 1959. The mechanism of formation of succinate and propionate by Ascaris lumbricoides muscle. J. Biochem. 234:2001-2005.

Schwabe, C.W. 1957. Observations on the respiration of free living and parasitic Nippostrongylus muris larvae. Amer. J. Hyg. 65: 325-337.

Seinhorst, J.W. 1950. De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaaltje (Ditylenchus dipsaci (Kuhn)(Filipjev). T. Pl. Ziekten 56:289-348.

Spector, W.S. 1956. Handbook of biological data. W. B. Saunders Co., Philadelphia. 584 p.

Steiner, G. 1952. The soil to its relationship to plant nematodes. Proc. Soil Soc. Florida 12:24-29.

Stolzy, L.H., Van Gundy, S.D., and Letey, J. 1960. Oxygen tolerances of four plant parasitic nematodes. Phytopathology 50:656.

Thorne, G. 1961. Principles of Nematology. McGraw Hill Book Company, Inc., New York. 553 p.

Tiner, J.D. 1960. Cultures of plant parasitic nematode genus Pratylenchus on sterile excised roots. I. Their establishment and maintenance. Exptl. Parasitol. 9:121-126.

Van Gundy, S.D. 1965. Factors in survival of nematodes. Ann. Rev. Phytopath. 3:43-68.

Van Gundy, S.D. and Stolzy, L.H. 1961. Influence of soil oxygen concentrations on the development of Meloidogyne javanica. Science 134:665-666.

Van Gundy, S.D. and Stolzy, L.H. 1963. The relationship of oxygen diffusion rates to the survival, movement and reproduction of Hemicycliophora arenaria. Nematologica 9:605-612.

_____, _____, Szuszkiewicz, T. and Rackham, R.L. 1962. Influence of oxygen supply on survival of plant parasitic nematodes in soil. Phytopathology 52:628-632.

von Brand, T. 1942. Physiological observations upon a larval Eustrongyloides. II. The aerobic respiration. Biol. Bull. 82:1-13.

_____. 1943. Physiological observations upon a larval Eustrongyloides. IV. Influence of temperature, pH and inorganic ions upon the oxygen consumption. Biol. Bull. 84:148-156.

_____. 1952. Chemical physiology of endoparasitic animals. Academic Press Inc., New York. 339 p.

_____. 1960. Influence of pH, ions, and osmotic pressure on life processes. p. 240-256. In: Nematology. Fundamentals and recent advances with emphasis on plant parasitic and soil forms. Editors: Sasser, J.N. and Jenkins, W.R. The University of North Carolina Press, Chapel Hill.

Wallace, H.R. 1958. Movement of eel-worms. 2. A comparative study of the movement in soil of Heterodera schachtii Schmidt and of Ditylenchus dipsaci (Kuhn) Filipjev. Ann. Appl. Biol. 46:86-94.

_____. 1959. Further observations on some factors influencing the emergence of larvae from cysts of the beet eelworm, Heterodera schachtii Schmidt. Nematologica 4:245-252.

_____. 1961. The orientation of Ditylenchus dipsaci to physical stimuli. Nematologica 6:222-236.

_____. 1963. The biology of plant parasitic nematodes. Edward Arnold Ltd., London. 280 p.

Wallace, H.R. and Greet, D.N. 1964. Observations on the taxonomy and biology of Tylenchorhynchus macrurus (Goodey, 1932) Filipjev, 1936 and Tylenchorhynchus icarus sp. nov. Parasitology 54:129-144.

Warren, C.O. 1944. The role of bicarbonate in the action of serum in supporting tissue respiration. J. Biol. Chem. 156:559-569.

Warren, L.G., Guevara, A. and Patzrek, D. 1962. Respiration and carbohydrate metabolism of Ancylostoma caninum. Effect of carbohydrates, acid intermediates and electron transport inhibitors. J. Parasitol. 48:25.

Winslow, R.D. 1960. Some aspects of ecology of free living and plant parasitic nematodes. p. 341-415. In: Nematology. Fundamentals and recent advances. Editors: Sasser, J.N. and Jenkins, W.R. The University of North Carolina Press. Chapel Hill.

APPENDIX

ANALYSIS OF VARIANCE TABLES

TABLE i. Analysis of variance of Q_{O_2} values of Pratylenchus penetrans at osmotic pressures -- 0, 0.224, 2.24, 22.4 and 44.8 atmospheres.

Source of variation	degrees of freedom	sum of squares	Mean squares
Treatments	4	40.701	8.140
Within treatments	20	2.282	0.114
Total	24	42.983	

Correction term = 590.765

$S\bar{d}$ = 0.213

SE = $\pm$0.153

Least significant difference - at 95% confidence level = 0.444

- at 99% confidence level = 0.605

TABLE ii. Analysis of variance of Q_{O_2} values of D. dipsaci at osmotic pressures -- 0, 0.224, 2.24, 22.4 and 44.8 atmospheres.

Source of variation	Degrees of freedom	sum of squares	Mean squares
Treatments	4	78.868	19.717
Within treatments	20	2.573	0.128
Total	24	81.441	

Correction term = 306.509

$S\bar{d}$ = 0.225

SE = $\pm$0.160

Least significant difference - at 90% confidence level = 0.389

- at 95% confidence level = 0.471

TABLE iii. Analysis of variance of Q_{O_2} values of Anguina agrostis larvae at osmotic pressures -- 0, 0.224, 2.24, 22.4 and 44.8 atmospheres.

Source of variation	degrees of freedom	sum of squares	Mean squares
Treatments	4	3.119	0.779
Within treatments	20	3.870	0.193
Total	24	6.989	

Correction term = 81.352

$S\bar{d}$ = 1.277

SE = $\pm$0.301

Least significant difference - at 90% confidence level = 0.479

- at 95% confidence level = 0.579

TABLE iv. Analysis of variance of Q_{O_2} values of Anguina tritici larvae at 0, 0.224, 2.24, 22.4 and 44.8 atmospheres osmotic pressures.

Source of variation	degrees of freedom	sum of squares	Mean squares
Treatments	4	1.280	0.320
Within treatments	15	0.530	0.035
Total	19	1.910	

Correction term = 31.465

$S\bar{d}$ = 0.132

SE = $\pm$0.093

Least significant difference - at 95% confidence level = 0.281

- at 99% confidence level = 0.389

TABLE v. Analysis of variance of Q_{O_2} values of Ditylenchus dipsaci at carbon dioxide concentrations -- 0, 0.03, 0.1, 0.5, 1.0 and 2.0 percent.

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Treatments	5	553.740	110.748
Within treatments	24	48.866	2.036
Total	29	602.606	

Correction term = 1704.221

$S\bar{d}$ = 0.902

SE = $\pm$0.638

Least significant difference - at 95% confidence level = 1.862

- at 99% confidence level = 2.522

TABLE vi. Analysis of variance of Q_{O_2} values of Aphelenchoides ritzemabosi at carbon dioxide concentrations -- 0, 0.03, 0.1, 0.5, 1.0 and 2.0 percent.

Source of variation	Degrees of freedom	sum of squares	Mean squares
Treatments	5	1472.019	294.403
Within treatments	24	417.287	17.386
Total	29	1189.306	

Correction term = 4771.397

$S\bar{d}$ = 2.637

SE = ±1.848

Least significant difference - at 95% confidence level = 5.442

- at 99% confidence level = 7.375

TABLE vii. Analysis of variance of Q_{O_2} values of P. penetrans at carbon dioxide concentrations -- 0, 0.03, 0.1, 0.5, 1.0 and 2.0 percent.

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Treatments	5	168.468	33.693
Within treatments	24	159.738	6.655
Total	29	328.206	

Correction term = 2014.544

$S\bar{d}$ = 1.631

SE = $\pm$1.153

Least significant difference - at 90% confidence level = 2.790

- at 95% confidence level = 3.336

- at 99% confidence level = 4.561